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A phase I, first in human study of FP-1039 (GSK3052230), a novel FGF ligand trap, in patients with advanced solid tumors

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Background: Fibroblast growth factors (FGFs) play important roles in multiple cancers by supporting tumor growth and angiogenesis. FP-1039 (GSK3052230) is a FGF ligand trap consisting of the extracellular domain of FGF receptor 1 (FGFR1) fused with the Fc region of IgG_1. FP-1039 binds and neutralizes multiple FGFs that normally bind FGFR1. The primary objective of this phase I study was to evaluate the safety and tolerability of FP-1039.

Patients and methods: Eligible patients with metastatic or locally advanced solid tumors for which standard treatments were ineffective were treated with weekly doses of FP-1039 for 4 weeks, followed by 2 weeks observation.

Results: Thirty-nine subjects received a mean of 6 infusions of FP-1039 at doses ranging from 0.5 to 16 mg/kg weekly, with no maximally tolerated dose identified. Grade 3 or greater treatment emergent adverse events were uncommon. Four dose-limiting toxicities were reported at doses of 0.75 mg/kg (urticaria), 1 mg/kg (intestinal perforation and neutropenia), and 16 mg/kg (muscular weakness). Drug exposure was dose proportional, and the terminal elimination half-life was 2.6–3.9 days following a single dose. Target engagement as measured by low free plasma FGF2 levels was achieved. FGF pathway dysregulation was uncommon. No objective responses were observed.

Conclusion: In nonselected cancer patients with advanced disease, treatment with FP-1039 was well tolerated and toxicities associated with small molecule drugs that inhibit FGFR tyrosine kinases, including hyperphosphatemia, were not observed. Further studies of FP-1039 in patients selected for FGF pathway dysregulation, who are most likely to benefit, are now underway.

Key words: cancer, FGF, ligand trap, phase I

introduction

Fibroblast growth factors (FGFs) are a large family of secreted growth factors that bind to a set of 4 cognate receptor tyrosine kinases (FGFR 1–4). The 22-member FGF family comprises 18 distinct proteins that bind with varying affinities to the FGFRs and 4 structurally related proteins that may not bind FGFRs [1, 2]. Most FGFs function as stimulators of cellular proliferation, survival and differentiation, tissue development, angiogenesis, and wound healing. Several of the FGFs, however, function instead as hormones. For example, FGF23 is thought to be a principle regulator of serum phosphate homeostasis [3].

The FGF pathway, and in particular FGF2 and FGFR1, have been extensively implicated in cancer. FGFR signaling stimulates tumor angiogenesis and growth and increases the potential of tumor development from cancer stem cells [4]. Mutations, copy number alterations, and chromosomal translocations of FGFs and FGFRs are observed in multiple tumor settings. For example, amplification of the *FGFR1* gene occurs in a number of tumors and is associated with poor survival in patients [5–9]. Additionally, overexpression of FGF ligands, most notably FGF2, is correlated with tumorigenesis and poor patient outcome [10].

Based on the role of the FGF–FGFR signaling in cancer, agents that inhibit this pathway are attractive as potential new cancer treatments. Blockade of FGFR1 signaling using monoclonal antibodies directed against FGFR1 proved toxic in animal studies and no anti-FGFR1 antibodies have been advanced to the clinic [11]. Small molecule inhibitors of FGFR1 tyrosine kinase activity inhibit FGF-driven mitogenesis [12]. However,

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the kinase domains are relatively conserved between the different FGFRs, and current tyrosine kinase inhibitors are generally nonselective among the different FGFRs. As a consequence, FGFR tyrosine kinase inhibitors have displayed toxicities that are thought to reflect broad inhibition of the entire FGF family, notably including dose-limiting hyperphosphatemia due to inhibition of the hormonal FGF, FGF23 [13–15].

FP-1039 was developed as a soluble fusion of the extracellular, ligand-binding domain of FGFR1 linked to a modified hinge and native Fc regions of human immunoglobulin G1 (IgG1). This fusion protein selectively binds to and neutralizes multiple FGF ligands that normally bind FGFR1—a so-called FGF ligand trap [16]. The binding specificity of the extracellular domain portion of FP-1039 appears to be similar to that of the endogenous FGFR1 [16]; therefore, FP-1039 sequesters FGF ligands that bind to FGFR1 and inhibits their activation of FGFR1. Importantly, FP-1039 does not effectively inhibit FGF23, which requires the co-receptor Klotho for binding and downstream activity [17, 18], and FP-1039 does not produce hyperphosphatemia in preclinical studies [16]. Therefore, FP-1039 has the potential to be used at doses that more effectively block the cancer-promoting FGFs, and with less toxicity, than small molecule FGFR kinase inhibitors.

In preclinical models, FP-1039 blocked FGF2-stimulated tumor cell proliferation and inhibited tumor growth in 19 of 35 xenograft models tested, including models of *FGFR1*-amplified lung cancer and FGF2-overexpressing mesothelioma [16, 19].

Based on these data, a first-in-human study was conducted in which FP-1039 was tested in patients with solid tumors. The primary objective was to evaluate the safety and tolerability of FP-1039. Additionally, the pharmacokinetic (PK) and pharmacodynamic profiles of FP-1039 were characterized, and preliminary antitumor activity was assessed.

patients and methods

general

This was a phase I, open-label, ascending-dose study designed to assess the safety, tolerability, PK, and target engagement of intravenous (i.v.) FP-1039 administered weekly to adult patients with metastatic or locally advanced unresectable solid tumors. Eligible patients were ≥18 years of age and had histologically or cytologically proven metastatic or locally advanced unresectable solid tumors for which standard curative or palliative measures did not exist or were no longer effective. Additional eligibility requirements included an Eastern Cooperative Oncology Group (ECOG) performance score of 0–2 and the presence of measurable or evaluable disease. Patients with prior therapy were to have recovered from, and to have been at least 4 weeks free from, previous chemotherapy and at least 4 weeks or 4 half-lives (whichever was longer) removed from previous biologics therapy. Patients with a history of melanoma or having a current melanoma were not eligible for study participation. The study was approved at each site by a local institutional review board (IRB) and all patients signed a written informed consent before participation in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and with the International Conference on Harmonization Good Clinical Practice Guideline and registered as NCT00687505.

drug administration

Patients received FP-1039 once weekly for a total of 4 i.v. infusions (30-min each) over 3 weeks, followed by a 2-week observation period. A 3 + 3 trial design was incorporated with the dose-limiting toxicity (DLT) rate not to exceed 1/6 patients. Dose escalation occurred after an acceptable safety profile was assured on the day 36 visit. Enrollment into ascending-dose cohorts occurred until either the maximum tolerated dose (MTD) or the maximum feasible dose was reached.

Patients with no evidence of disease progression or DLT could continue to receive additional weekly infusions of FP-1039 until disease progression or toxicity occurred, and end-of-study visits occurred ∼4 weeks (±1 week) after the last dose of FP-1039. Dose levels (cohorts) of FP-1039 evaluated in this study were 0.5, 0.75, 1, 2, 4, 8, and 16 mg/kg.

pretreatment and follow-up studies

Physical examination, ophthalmic assessments, vital signs assessments, 12-lead electrocardiogram (ECG), echocardiogram, clinical laboratory sampling, radiological tumor assessments, and ECOG performance status were performed at enrollment. The safety of FP-1039 was assessed by monitoring adverse events (AEs) and changes in these parameters during the safety assessment.

Assessment of toxicities followed the guidelines in the National Cancer Institute (NCI)—Common Terminology Criteria for Adverse Events (CTCAE), version 3.0, and a study-specific anterior uveitis grading scale.

A DLT was defined as any study drug-related, grade 3 or worse toxicity, including hematologic toxicities, except for asymptomatic grade 3 serum magnesium or phosphate levels of <2 weeks duration in patients with prior cetuximab or panitumumab exposure. Further, any grade 3 or worse anterior uveitis, regardless of relationship to study drug, was defined as a DLT.

plasma pharmacokinetic sampling and assay

Plasma concentrations of FP-1039 were evaluated at 0.5, 1, 2, 4, 8, 24, 48, 72, and 120 h following the first (day 1) and final fourth (day 22) doses. Concentrations of FP-1039 were determined using a validated FGF2 ligand-binding, enzyme-linked immunosorbent assay in accordance with FDA's published recommendations [20].

Plasma FP-1039 PK parameters were estimated from the plasma drug concentration–time data using noncompartmental analysis in WinNonlin (Version 5.2, Pharsight, Corp.). Area under the concentration–time curve (AUC) from time 0 to time τ, the end of the dose interval, was calculated by the linear trapezoidal method. Maximal plasma concentration (C_{max}) was the observed value, and elimination half-life ($t_{1/2}$) was calculated from the apparent first-order terminal elimination rate constant. C_{max} and AUC were normalized (C_{max}/D and AUC/D) by the administered dose (mg/kg).

Plasma was also tested for anti-FP-1039 antibodies (antidrug antibodies, ADAs). Plasma samples were obtained before and during the treatment period, at the end of the observation period, and every 3 months during the extended treatment period. ADAs were determined using a validated electrochemiluminescence bridging assay following published recommendations [21].

plasma pharmacodynamic sampling and assay

Blood samples were collected for measurement of FGF2 plasma concentrations by a modified commercial immunoassay (Meso Scale Discovery, Rockville, MD) that measures free FGF2 which is not bound to FP-1039.

tumor assessments

Tumor assessments were performed at screening, on day 36 (range day 30–37, but before extended treatment), every 8 weeks during the extended treatment period, and at the end-of-study visit. Response was evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) for those with measurable disease [22], and tumor markers where appropriate. Fludeoxyglucose positron emission tomography (FDG PET) scans were incorporated to assess changes in tumor metabolic activity before treatment and on ∼day 15; FDG PET scans were repeated on day 56 for those patients who received at least seven infusions.

Archival 5-μm formalin-fixed and paraffin-embedded (FFPE) tumor sections from a subset of subjects were assessed for FGF2 and FGFR1 protein expression by immunohistochemistry (IHC) and for *FGFR1* gene amplification by fluorescent *in situ* hybridization (FISH). See supplementary Material, available at *Annals of Oncology* online.

results

general

Thirty-nine subjects with advanced solid tumors refractory to standard therapy were enrolled between August 2008 and July 2011, with the pertinent demographics listed in Table 1. Patients received a median of 4 and a mean of 6 FP-1039 infusions (ranging from 1 to 26 infusions).

Table 1. Patient characteristics

Characteristic	Number of patients
Number of patients	39
Number of courses/patient	
Mean, median (range)	5.9, 4 (1–26)
Age, years	
Median (range)	64.0 (27–77)
Sex	
Male	15 (38.5%)
Female	24 (61.5%)
No. of prior therapy regimens	
Median (range)	4 (0–11)
Tumor type	
Breast cancer	7 (19.9%)
Colon/rectal cancer	5 (12.8%)
Head and neck cancer	4 (10.3%)
Hepatobiliary cancer	2 (5.1%)
Neuroendocrine tumors	1 (2.6%)
Nonsmall-cell lung cancer	3 (7.7%)
Pancreatic adenocarcinoma	1 (2.6%)
Prostate cancer	5 (12.8%)
Sarcoma	2 (5.1%)
Small bowel cancer	1 (2.6%)
Soft tissue sarcoma	4 (10.3%)
Uterine neoplasm	4 (10.3%)

Seven dose levels were assessed in eight treatment cohorts. The first cohort (cohort 1) of three patients received 1 mg/kg FP-1039; however, two DLTs were observed in two patients in this cohort: neutropenia (grade 3) and gastrointestinal (GI) perforation. The neutropenia occurred in a 52-year-old male with metastatic prostatic adenocarcinoma who had been previously treated with docetaxel. The GI perforation occurred in a 64-year-old female with malignant liposarcoma of the retroperitoneum, which had been treated by three independent surgical resections and adjuvant abdominal radiotherapy. A CT scan conducted at the time of the GI perforation event confirmed extensive tumor involvement of the bowel.

The protocol was subsequently amended to exclude patients with risk factors for GI perforation. The study progressed at a decreased dose of 0.5 mg/kg in six patients (cohort 1). As there were no DLTs in this cohort, a dose of 0.75 mg/kg was administered to six patients (cohort 2) with one grade 2 DLT (urticaria); the 1 mg/kg dose was then re-evaluated in another three patients (cohort 1B). No DLTs were observed in cohort 1B and higher FP-1039 doses were then evaluated at 2 mg/kg (cohort 2B), 4 mg/kg (cohort 3B), 8 mg/kg (cohort 4B), and 16 mg/kg (cohort 5B), with a single DLT identified in cohort 5B (muscular weakness). 16 mg/kg was designated the maximum feasible dose, and no MTD was identified.

adverse events

During treatment with FP-1039, the most frequently observed AEs were diarrhea (43.6%), fatigue (43.6%), and nausea (25.6%). In the 16 mg/kg dose cohort, nausea and vomiting were observed in 50 and 40% of patients, respectively, although these were limited in severity (grade 1 or 2) and considered possibly or probably related to FP-1039 in only 20 and 10% of patients, respectively. The most commonly reported grade 3 toxicities across all dose groups were hyperglycemia, hypokalemia, hypophosphatemia, abdominal pain, and dyspnea, each reported in two (5.1%) patients (Table 2).

There were seven (17.9%) patients that discontinued from the study due to a treatment emergent AE. There were no apparent trends in the number of discontinuations across cohorts, with one patient discontinued from each of five treatment cohorts,

Table 2. Treatment-related toxicities as a function of dose level

System organ class and MedDRA preferred term	Total number of subjects	Dosing cohort and CTCAE grade*																									
		0.5 mg/kg				0.75 mg/kg				1 mg/kg				2 mg/kg				4 mg/kg				8 mg/kg				16 mg/kg			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Leukopenia	2																										1		
Neutropenia	1											1																	
Diarrhea	11	3				2					2							1				2				1			
Nausea	6	1				1					1			1												2			
Vomiting	2					1																					1		
Fatigue	11	1	2			1				2				1				2									2		
Edema peripheral	3									1	1															1			
Headache	3					1																				2			
Pruritus	2	1				1																							
Rash	2	1																								1			

*NCI CTC AE V 3.0.

and two patients discontinued from cohort 1. There were six patients (15.4%) that had a treatment emergent AE that led to study drug interruption. Study drug interruption occurred in one patient each of cohorts 1, 3B, and 4B. In cohort 5B, study drug interruption occurred in three patients.

No clinically significant drug-related ECG and echocardiograms were observed.

pharmacokinetics

Mean plasma FP-1039 concentration time profiles for the first (day 1) and final, fourth (day 22) infusions are presented in Figure 1. Plasma FP-1039 concentrations were maintained above the lower limit of assay quantitation for at least 168 h in each dose group following dose administration. After day 1 infusion, the terminal half-life ($t_{1/2}$) across all the dose cohorts, was a mean value of 2.6–3.9 days after the first dose and 3.6–5.2 days after the fourth dose (Table 3). Dose-normalized values for C_{max} and AUC (C_{max}/D and AUC/D) were similar among the different dose cohorts, demonstrating that both parameters increased in a dose-proportional manner (Table 3). Accumulation of FP-1039 was evident in plasma over the course of the dosing period (4 weekly i.v. doses) when comparing the PK data following the first dose with that following the fourth dose. Mean trough plasma concentration (C_{min}, concentration at 168 h post-dose) increased after each of the four infusions. Mean C_{max} following the fourth infusion was 1.2–1.4 times higher than the mean C_{max} following the first infusion, and mean AUC following the fourth infusion was 1.4–2.2 times higher than the mean AUC after the first infusion (Table 3). Comparison of PK parameters by two-way ANOVA showed no significant effects of gender among treatment cohorts (data not shown).

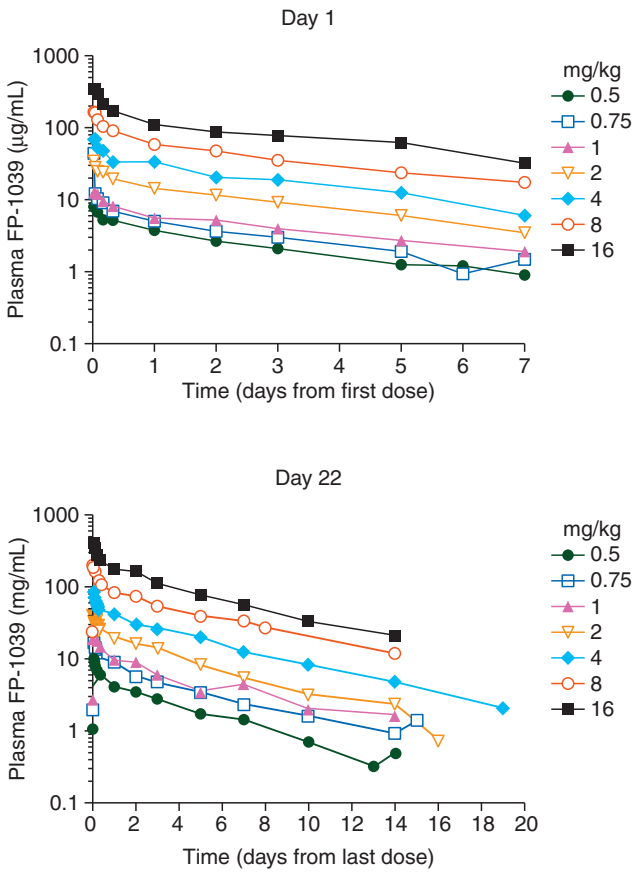

Figure 1. Mean plasma FP-1039 concentration versus time profiles following 30 min i.v. infusion of escalating doses on day 1 and day 22.

immunogenicity

Eleven of the 36 patients evaluated for anti-FP-1039 antibodies (ADAs) were positive, most commonly on day 15. Two patients had pre-existing ADAs before FP-1039 dosing. Nine of the 11 ADA-positive subjects tested negative for ADAs by day 39, suggesting the response was a transient primary (IgM) response that did not lead to isotype switching. The remaining two ADA-positive subjects continued to test positive during extended treatment. The ADA response was generally weak (low titer) with no apparent associated changes in drug exposure or clinical sequelae.

pharmacodynamics

Changes in plasma FGF2 concentrations following FP-1039 administration were considered an indicator of drug target engagement as this assay measures FGF2 not bound to FP-1039. Free FGF2 was detected (>2 pg/ml) in 37 of 39 (95%) patients before dosing on day 1, when compared with no detectable FGF2 in any plasma samples from 35 healthy donors tested by the same method (Figure 2) [10]. Following FP-1039 treatment, free FGF2 concentrations decreased on days 2, 8, 15, and 36 for all dose cohorts (Figure 2 and data not shown). The decrease was statistically significant (one-way ANOVA with Tukey post-test, $P < 0.05\%$) on day 15 for the 0.75 mg/kg dose cohort, and on days 2, 8, 15, and 36 for the 1 and 16 mg/kg cohorts. The combined data from all cohorts showed a decrease ($P < 0.001$) on all post-dose days.

Table 3. Summary of PK parameters for FP-1039 following intravenous administration

PK parameters (units)	FP-1039 dose (mg/kg)						
	0.5	0.75	1	2	4	8	16
Following first infusion (mean values)							
AUC (µg h/ml)	378	567	612	1630	3189	7181	14 028
AUC/D	755	756	612	815	797	898	877
C_{max} (µg/ml)	8.5	13.3[a]	13.8	34.7	75.3	176.4	372
C_{max}/D	17	18	14	17	19	22	23
$t_{1/2}$ (days)	3.0	3.9	3.4	3.0	2.6	3.7	3.8
Following fourth infusion (mean values)							
AUC (µg h/ml)	615	835	1317	2413	4882	10 173	21 149
C_{max} (µg/ml)	12.2	17.4	19.5	44.8	90.9	202.9	443.1
$t_{1/2}$ (days)	3.6	4.3	4.5	3.7	4.1	4.5	5.2
Comparison of first versus fourth infusion[b]							
AUC_4/AUC_1	1.6	1.5	2.2	1.5	1.5	1.4	1.5
C_{max4}/C_{max1}	1.4	1.3	1.4	1.3	1.2	1.2	1.2

[a]The observed C_{max} for one of six subjects in this cohort was an outlier and was removed from the analysis.
[b]Data following the fourth infusion were divided by the data following the first infusion.

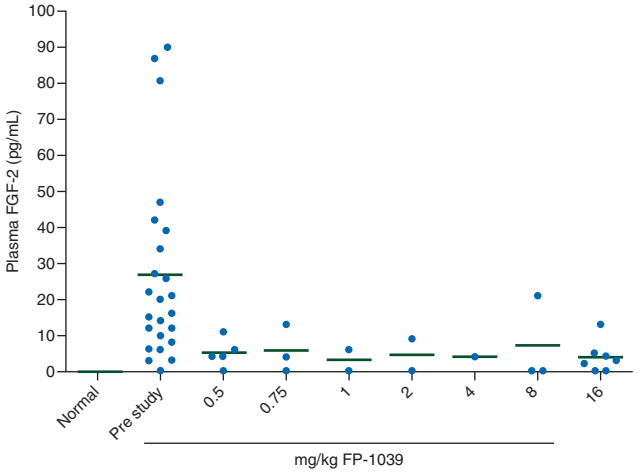

Figure 2. Free FGF-2 levels in plasma. FGF2 levels predose and at 14 days post last dose by cohort compared with normal subjects.

The mean (± standard deviation) free FGF2 value across all cohorts on day 1 (before dosing) was 32.4 (±37.3 pg/ml) compared with 3.7 (±3.4 pg/ml), 5.1 (±4.0 pg/ml), 2.9 (±3.5 pg/ml), and 4.7 (±5.5 pg/ml) on days 2, 8, 15, and 36, respectively.

efficacy

No objective responses were observed in this study. One patient with castrate-resistant prostate cancer had a 20% decrease in tumor measurements, a corresponding decrease in FDG PET standardized uptake value, and stable disease for 7 months. In the subgroup of patients with measurable disease, 15 subjects (41.7%) had a best response of stable disease, and 17 subjects (47.2%) had progressive disease.

characterization of FGF pathway components in archival tumor tissue

Archival tumor samples from 11 patients were available for analysis of FGF2 and FGFR1 protein expression by IHC, and *FGFR1* gene amplification by FISH. Five subjects' tumors showed no evidence of FGF2 or FGFR1 expression by IHC or amplification of the *FGFR1* gene (data not shown). Generally, in these patients who were not selected for FGF pathway dysregulation, FGFR1 protein expression or gene amplification were at low levels, and high-level expression of FGF2 was infrequent. There was no apparent relationship between best tumor response and the presence of altered FGF pathway components in this small sample of patients (data not shown).

discussion

The presence of gene amplifications, activating point mutations, and chromosomal translocations in the FGF–FGFR pathway across a spectrum of human malignancies strongly suggests that inhibitors of FGF signaling may be useful new treatments for patients bearing tumors with these genetic alterations [5, 8, 23–25]. However, effectively targeting the FGF–FGFR pathway has been difficult in the past. Treatment of rodents and monkeys with monoclonal antibodies targeting FGFR1 resulted in severe weight loss, associated with hypothalamic binding of the antibody [11]. Further, while small molecule inhibitors of FGFR tyrosine kinases (TKIs) are effective in preclinical tumor models [12], their human use has been limited by toxicities from indiscriminate inhibition of all FGF signaling, including signaling required for normal physiologic functions. For example, inhibition by FGFR TKIs of FGF23, a hormone that regulates serum phosphate levels, causes hyperphosphatemia and calcium–phosphorus deposition in soft tissues in rats [26], and in humans, causes hyperphosphatemia and renal failure [13–15].

FP-1039 is a novel approach to inhibiting the FGF pathway that does not suffer from the same limitations. It consists of a FGFR1 fusion protein that inhibits the activity of mitogenic, cancer-associated FGFs, without inhibiting the hormonal FGFs, particularly FGF23 [16]. The results of this phase I study established an acceptable human safety profile for FP-1039. While all patients treated with FP-1039 experienced an AE, most were not severe and many unrelated to study drug. Treatment with FP-1039 was well tolerated over the dose range studied (0.5–16 mg/kg) with few DLTs, and a protocol-defined MTD was not reached. Importantly, there were no reports of toxicities that have been associated with FGFR1 antibodies or FGFR TKIs, such as marked weight loss, hyperphosphatemia, skin and nail bed changes, retinal detachment or oral pain. Since no MTD was reached, the highest dose examined (16 mg/kg) was determined to be the maximum feasible dose. Following the completion of this trial, FP-1039 was renamed GSK3052230, and the methodology for determining drug concentration changed, such that the 16 mg/kg maximum feasible dose reported in this study is equivalent to 20 mg/kg reported in future studies.

This trial also provides data demonstrating that the pharmaceutical properties of FP-1039 are adequate for further study in patients. Immunogencity of FP-1039 was minimal in this trial. Plasma levels of FP-1039 were approximately dose proportional following a single i.v. dose and were maintained after 4 weekly i.v. doses ranging from 0.5 to 16 mg/kg. Some accumulation of FP-1039 was observed after 4 weekly doses suggesting that less frequent dosing intervals could be explored in subsequent trials.

Because the presence of serum FGF2 is associated with poor prognosis in multiple tumor types, changes in plasma FGF2 were used as a relevant pharmacodynamic biomarker of target engagement. FP-1039 administration resulted in target engagement as measured by a significant decrease in free FGF2 levels in plasma across all dose levels. The data in Figure 2 and in assay validation experiments (see Patients and methods section) suggest that 100% target engagement in the plasma had been achieved in most subjects at all dose levels.

No objective responses were observed in this unselected population of patients with advanced, refractory solid tumors that are unlikely to be driven by dysregulated FGF–FGFR signaling. The greatest potential for FP-1039 is in the treatment of tumors that are dependent on the FGF pathway for growth and survival due to alterations in the FGF pathway components. For example, the *FGFR1* gene resides in the short arm of chromosome 8, which is a frequent target of genetic alterations [27, 28]. A focal amplicon that contains *FGFR1* as the only amplified gene is observed in ~7%–22% of squamous nonsmall-cell lung carcinoma (SqNSCLC) [5]. Preclinically, SqNSCLC cells and tumor models bearing the *FGFR1* amplification are sensitive to FP-1039 [16, 29]. This is consistent with the observation that cells bearing the amplified *FGFR1* gene remain ligand-dependent and

therefore would be predicted to respond to a FGF ligand trap [30]. Further, some responses to single-agent FGFR TKI therapy have been reported in patients with *FGFR1* amplifications, despite limitations in dosing due to toxicities [13, 15]. No subjects with *FGFR1*-amplified SqNSCLC were identified in this phase I trial, but three subjects with *FGFR1* gene amplification in other tumor types were identified. However, the level of amplification was low, and its relevance in other tumor types, when found within larger amplicons that contain other amplified genes, has been questioned. For example, in breast cancer, the amplicon involves a large region of the chromosome 8p11-12, including 14 genes, some with known role in tumor growth (e.g. CCND1) [8].

Tumors with alterations in expression of the FGF ligands are also attractive targets for FP-1039, especially since FP-1039 is a ligand trap. Many human tumors and cancer cell lines express high levels of FGF2, and as noted, serum FGF2 levels correlate with poor prognosis in many different tumors. Although serum levels of FGF2 were elevated in the patient population in this study, high-level FGF2 overexpression in tumors by IHC was infrequent in this trial, and no correlation with best tumor response was observed in this small number of patients. A potentially attractive tumor type to study in the future with FP-1039 in this respect is mesothelioma, which was not studied in this trial. Mesothelioma cell lines express among the highest levels of FGF2 of all cancer cell lines, and mesothelioma xenograft models are sensitive to FP-1039 [16, 19].

In conclusion, this phase I trial demonstrates that FP-1039 has an acceptable safety profile and is well tolerated at the doses tested, with no MTD having been identified. Therefore, FP-1039 is expected to combine well with standard of care chemotherapy, and is an attractive alternative to FGFR TKIs or FGFR1 monoclonal antibodies for patients with tumors driven by FGF pathway alterations. To test FP-1039/GSK3052230 in a patient population most likely to benefit, a phase Ib trial is currently underway to assess the safety, PK, and preliminary efficacy of FP-1039 in patients with either *FGFR1* gene-amplified SqNSCLC or mesothelioma in combination with standard chemotherapy (NCT01868022).

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disclosure

JZ, ATG, WMK, ST and HNK are/were employees of Five Prime Therapeutics, Inc., at the time this study was performed. PML has been a consultant for Five Prime Therapeutics, Inc. AWT is a consultant/advisory board member for AbbVie, Adnexus, Ambit, AP Pharma, Aragon, Ariad, ArQule, Astellas, Astellas Japan, Astex, Bayer, Bind, BioMed Valley Discoveries, Blend Therapeutics, Bristol-Myers-Squibb Japan, Celator, Clovis, Curis, Daiichi Sankyo, Dicerna, Eisai, Emergent Product Development, Five Prime, Galapagos, Janssen R&D, Eli Lilly, MedImmune, Merck Sharp & Dohme, Merus, Micromet, Nanobiotix, Nektar, Neumedicines, Nexus, Novartis, OncoGenex, Onyx, Otsuka, Pfizer, Pharmacyclics, Pierre Fabre, ProNai, Proximagen, Sanofi-Aventis, Santaris, Symphogen, Vaccinex, and Ztyngenia, and reports receiving fees for the above-mentioned associations through South Texas Accelerated Research Therapeutics, of which he is a co-owner. All remaining authors have declared no conflicts of interest.

references

1. Itoh N. The Fgf families in humans, mice, and zebrafish: their evolutional processes and roles in development, metabolism, and disease. Biol Pharm Bull 2007; 30: 1819–1825.
2. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov 2009; 8: 235–253.
3. Long YC, Kharitonenkov A. Hormone-like fibroblast growth factors and metabolic regulation. Biochim Biophys Acta 2011; 1812: 791–795.
4. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010; 10: 116–129.
5. Weiss J, Sos ML, Seidel D et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010; 2: 62ra93.
6. Dutt A, Ramos AH, Hammerman PS et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011; 6: e20351.
7. Turner NC, Seckl MJ. A therapeutic target for smoking-associated lung cancer. Sci Transl Med 2010; 2: 62ps56.
8. Gelsi-Boyer V, Orsetti B, Cervera N et al. Comprehensive profiling of 8p11–12 amplification in breast cancer. Mol Cancer Res 2005; 3: 655–667.
9. Chang J, Liu X, Wang S et al. Prognostic value of FGFR gene amplification in patients with different types of cancer: a systematic review and meta-analysis. PLoS ONE 2014; 9: e105524.
10. Nguyen M, Watanabe H, Budson AE et al. Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. J Natl Cancer Inst 1994; 86: 356–361.
11. Sun HD, Malabunga M, Tonra JR et al. Monoclonal antibody antagonists of hypothalamic FGFR1 cause potent but reversible hypophagia and weight loss in rodents and monkeys. Am J Physiol Endocrinol Metab 2007; 292: E964–E976.
12. Guagnano V, Furet P, Spanka C et al. Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamin o]-pyrimidin-4-yl}-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. J Med Chem 2011; 54: 7066–7083.
13. Paik PK, Shen R, Ferry D et al. A phase 1b open-label multicenter study of AZD4547 in patients with advanced squamous cell lung cancers: preliminary antitumor activity and pharmacodynamic data. J Clin Oncol 2014; 32(suppl): abstr 8035.
14. Bahleda R, Dienstmann R, Adamo B et al. Phase 1 study of JNJ-42756493, a pan-fibroblast growth factor receptor (FGFR) inhibitor, in patients with advanced solid tumors. J Clin Oncol 2014; 32(suppl): abstr 2501.
15. Nogova L, Lecia V, Sequist LV et al. Targeting FGFR1-amplified lung squamous cell carcinoma with the selective pan-FGFR inhibitor BGJ398. J Clin Oncol 2014; 32 (suppl): abstr 8034.
16. Harding TC, Long L, Palencia S et al. Blockade of nonhormonal fibroblast growth factors by FP-1039 inhibits growth of multiple types of cancer. Sci Transl Med 2013; 5: 178ra39.
17. Kurosu H, Ogawa Y, Miyoshi M et al. Regulation of fibroblast growth factor-23 signaling by klotho. J Biol Chem 2006; 281: 6120–6123.
18. Urakawa I, Yamazaki Y, Shimada T et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 2006; 444: 770–774.
19. De Young MP, Sherk C, Bleam M. et al. Preclinical efficacy of targeting FGF autocrine signaling in mesothelioma with the FGF ligand trap, FP-1039/GSK3052230. In Proceedings of the 105th Annual Meeting of the American Association for Cancer Research, 5–9 April 2014; 2014. Abstract LB-236, p. 74. AACR, Cancer Res, San Diego, CA; Philadelphia, PA.

20. U.S. Department of Health and Human Services FaDA, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM). Guidance for Industry: Bioanalytical Method Validation. 2001.
21. Mire-Sluis AR, Barrett YC, Devanarayan V et al. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J Immunol Methods 2004; 289: 1–16.
22. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45: 228–247.
23. Peifer M, Fernandez-Cuesta L, Sos ML et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012; 44: 1104–1110.
24. Kwek SS, Roy R, Zhou H et al. Co-amplified genes at 8p12 and 11q13 in breast tumors cooperate with two major pathways in oncogenesis. Oncogene 2009; 28: 1892–1903.
25. Freier K, Schwaenen C, Sticht C et al. Recurrent FGFR1 amplification and high FGFR1 protein expression in oral squamous cell carcinoma (OSCC). Oral Oncol 2007; 43: 60–66.
26. Brown AP, Courtney CL, King LM et al. Cartilage dysplasia and tissue mineralization in the rat following administration of a FGF receptor tyrosine kinase inhibitor. Toxicol Pathol 2005; 33: 449–455.
27. Emi M, Fujiwara Y, Nakajima T et al. Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer, and lung cancer. Cancer Res 1992; 52: 5368–5372.
28. Knowles MA, Shaw ME, Proctor AJ. Deletion mapping of chromosome 8 in cancers of the urinary bladder using restriction fragment length polymorphisms and microsatellite polymorphisms. Oncogene 1993; 8: 1357–1364.
29. Harding TC, Palencia S, Wallace T et al. Preclinical efficacy of fibroblast growth factor ligand trap HGS1036 in lung carcinoma models with genomic amplification of FGFR1. In Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 31 March–4 April 2012; 2012. Abstract 1876, p. 72. AACR; Cancer Res, Chicago, IL; Philadelphia, PA.
30. Malchers F, Dietlein F, Schottle J et al. Cell-autonomous and non-cell-autonomous mechanisms of transformation by amplified FGFR1 in lung cancer. Cancer Discov 2014; 4: 246–257.

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Methanol-based fixation is superior to buffered formalin for next-generation sequencing of DNA from clinical cancer samples

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Background: Next-generation sequencing (NGS) of tumour samples is a critical component of personalised cancer treatment, but it requires high-quality DNA samples. Routine neutral-buffered formalin (NBF) fixation has detrimental effects on nucleic acids, causing low yields, as well as fragmentation and DNA base changes, leading to significant artefacts.

Patients and methods: We have carried out a detailed comparison of DNA quality from matched samples isolated from high-grade serous ovarian cancers from 16 patients fixed in methanol and NBF. These experiments use tumour fragments and mock biopsies to simulate routine practice, ensuring that results are applicable to standard clinical biopsies.

Results: Using matched snap-frozen tissue as gold standard comparator, we show that methanol-based fixation has significant benefits over NBF, with greater DNA yield, longer fragment size and more accurate copy-number calling using shallow whole-genome sequencing (WGS). These data also provide a new approach to understand and quantify artefactual effects of fixation using non-negative matrix factorisation to analyse mutational spectra from targeted and WGS data.

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